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Ureaplasma urealyticum and *Mycoplasma hominis* in chlamydial and non-chlamydial nongonococcal urethritis

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SUMMARY Urethral specimens from 726 patients with nongonococcal urethritis (NGU) were examined for *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*. Chlamydiae were isolated from 35·9% of ureaplasma-positive patients and from 36·5% of ureaplasma-negative patients. Ureaplasmas were isolated from 52·5% of chlamydia-positive patients and from 53·1% of chlamydia-negative patients, an observation which contrasts with that of some workers who have suggested that ureaplasmas are significantly associated with chlamydia-negative NGU. Furthermore, the numbers of ureaplasmas isolated from patients who did or did not harbour chlamydiae were not significantly different nor was there a particular association of ureaplasmas with chlamydia-negative NGU in patients experiencing their first episode of disease. In addition, *M. hominis* was not isolated more frequently from those from whom chlamydiae were or were not isolated. The only significant associations were the isolation of *M. hominis* from patients who were ureaplasma-positive and of ureaplasmas from those who were *M. hominis*-positive. These findings do not necessarily mitigate against ureaplasmas being responsible for some cases of chlamydia-negative NGU.

Introduction

The results of several studies suggest that strains of *Ureaplasma urealyticum* (ureaplasmas) are responsible for some cases of nongonococcal urethritis (NGU). The most compelling evidence for this belief has arisen from human intraurethral inoculation of ureaplasmas (Taylor-Robinson *et al.*, 1977) and from antibiotic trials (Prentice *et al.*, 1976), particularly those in which antibiotics which differentiate between chlamydiae and ureaplasmas have been used (Bowie *et al.*, 1976; Coufalik *et al.*, 1979). It has been suggested by some investigators that ureaplasmas are particularly associated with non-chlamydial NGU. This notion has been based by one group of workers (Wong *et al.*, 1977) on their finding that ureaplasmas were associated only with non-chlamydial urethritis and not with NGU as a whole and by another group (Bowie *et al.*, 1976) on their finding that ureaplasmas were more frequently isolated from patients who were not infected by chlamydiae than from those who were infected. Over the past few years we have examined both clinically and microbiologically more than 700 patients with NGU. *Chlamydia trachomatis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* organisms were sought, those of the latter two species being quantitatively determined. Because of the quantitative as well as qualitative assessment of the micro-organisms in such a large group of patients, we have been able to evaluate with confidence the inter-relationship among ureaplasmas, *M. hominis*, and chlamydiae in NGU.

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Material and methods

PATIENTS

Male patients attending clinics at two hospitals in Central London—namely St Mary's Hospital and the Westminster Hospital—and at two hospitals in or near the Greater London area—namely the Central Middlesex Hospital and Shrodells Hospital,

Watford—were examined. The diagnosis of non-gonococcal urethritis (NGU) was made by passing a bacteriological loop 2 cm into the urethra and Gram staining a smear of the discharge. If gonococci, yeasts, and trichomonads were not seen and 15 or more polymorphonuclear leucocytes were present in one microscope field (×800 magnification), the patient was adjudged to have NGU and further specimens for microbiological study were taken. Consecutive patients attending each hospital during the period of the study and fulfilling these criteria were included in the study.

COLLECTION OF SPECIMENS AND PROCEDURE

A swab or bacteriological loop was used to inoculate urethral discharge on to GC selective agar medium (Oxoid). These plates were incubated at 36°C in 2% CO_2 in air for 48 hours and examined for the presence of Gram-negative, oxidase-positive diplococci (gonococci). A sterile cottonwool-tipped ENT swab was then inserted about 2 cm into the meatus and expressed into 1·8 ml of mycoplasma liquid transport medium. After a maximum of three hours, these samples were stored at −70°C until tested. They were titrated in urea-containing medium for the detection of ureaplasmas and in arginine-containing medium for *Mycoplasma hominis* (Taylor-Robinson *et al.*, 1971). The presence of these micro-organisms was confirmed by subculture in liquid media and *M. hominis* was identified by the use of specific antiserum in the disc growth-inhibition test. A specimen, for chlamydial culture, was taken by passing a similar swab about 2-3 cm into the urethra. It was expressed into sucrose-phosphate transport medium and stored immediately in liquid nitrogen until inoculated into McCoy cell cultures (Darougar *et al.*, 1971). In some cases, instead of γ-irradiation, the cell monolayers were treated with either 30 μg/ml of 5-iodo-2′-deoxyuridine for three days before inoculation (Wentworth and Alexander, 1974) or with 1 μg/ml of cycloheximide immediately after inoculation (Ripa and Mårdh, 1977).

METHODS OF STATISTICAL ANALYSIS

The χ^2 test with Yates's correction was used for comparisons of proportions, and the two-sample Student's t test for comparison of means. Results were considered significant if $P \leqslant 0{\cdot}05$.

Results

The results of attempts to isolate chlamydiae, ureaplasmas, and *M. hominis* from the urethra of a total of 726 patients with NGU who attended clinics at four hospitals between 1974 and 1978 are presented in Table 1. Chlamydiae were isolated from 36·2% of patients, ureaplasmas from 52·9%, and *M. hominis* from 13·4%.

OCCURRENCE OF UREAPLASMAS OR *M. HOMINIS* IN THE PRESENCE AND ABSENCE OF CHLAMYDIAE

Isolation of ureaplasmas

Ureaplasmas were not isolated significantly more frequently from patients who did not harbour chlamydiae than from those who did, whether or not patients attending each individual clinic or all patients are considered (Table 2). Furthermore, as shown in Figure 1, there was no tendency to recover larger numbers of ureaplasmas from patients who were chlamydia-negative than from those who were chlamydia-positive. Thus, the mean titre among chlamydia-negative patients was $10^{3{\cdot}78}$ and among chlamydia-positive patients $10^{3{\cdot}53}$ ($t = 1{\cdot}7$, $P \approx 0{\cdot}09$).

When 265 patients experiencing their first attack of NGU are considered alone, ureaplasmas were isolated from 61 (57%) of 107 who harboured chlamydiae and from 90 (57%) of 158 who did not, there obviously being no significant difference (χ_1^2 0·014, $P = 0{\cdot}91$).

Isolation of M. hominis

M. hominis organisms were not isolated significantly more frequently from patients who did not harbour chlamydiae than from patients who did, whether or not patients attending each individual clinic or all

Table 1 *Isolation of micro-organisms from patients with nongonococcal urethritis*

		Patients from whom micro-organisms were isolated						
		Chlamydiae		*Ureaplasmas*		M. hominis		
Hospital clinic	*Date seen*	*No.*	%	*No.*	%	*No.*	%	*Total no. of patients*
Central Middlesex	1974–1975	48	33·3	86	59·7	15	10·4	144
Westminster	1976–1977	51	41·5	55	44·7	13	10·6	123
Shrodell (Watford)	1976–1977	38	29·0	64	48·8	14	10·7	131
Central Middlesex	1976–1977	94	42·9	123	56·2	46	21	219
St Mary's (Paddington)	1978	32	29·3	56	51·4	9	8·3	109
Total		263	36·2	384	52·9	97	13·4	726

Table 2 *Isolation of* Ureaplasma urealyticum *from patients who are and are not infected by* Chlamydia trachomatis *or by* Mycoplasma hominis

	% of ureaplasma-positive patients among those who are					
	Chlamydia trachomatis			Mycoplasma hominis		
Hospital clinic	*Positive*	*Negative*	*Probability**	*Positive*	*Negative*	*Probability**
Central Middlesex	50·0	64·5	0·145	100	55·0	0·002
Westminster	51·0	40·3	0·324	84·6	40·0	0·006
Shrodell (Watford)	42·1	51·6	0·425	92·9	43·6	0·001
Central Middlesex	59·6	53·6	0·458	87·0	48·0	<0·0005
St Mary's (Paddington)	50·0	51·9	0·941	100	47·0	0·007
Total	52·5	53·1	0·762	90·7	47·1	<0·0005

*P *is significant when* <0·05

patients are considered (Table 3). In addition, as shown in Figure 2, the recovery of large numbers of *M. hominis* organisms was not significantly associated with chlamydia-negative patients. Thus, the mean titre among chlamydia-negative patients was $10^{3\cdot03}$ and among chlamydia-positive patients $10^{2\cdot63}$ ($t=1\cdot63$, P=0·12).

When patients experiencing a first episode of NGU are considered alone, *M. hominis* organisms were isolated from 16 (15%) of 107 who harboured chlamydiae and from 31 (19·6%) of 158 who did not harbour them, a difference which was also not significant (χ_1^2 0·659, P=0·41).

OCCURRENCE OF CHLAMYDIAE IN THE PRESENCE AND ABSENCE OF UREAPLASMAS OR *M. HOMINIS*

Chlamydiae were not isolated significantly more frequently from patients who did not harbour ureaplasmas than from those who did (Table 4), irrespective of whether or not patients attending each individual clinic or all patients are considered. The same conclusions were drawn about the occurrence of chlamydiae in patients who were or were not infected by *M. hominis* (Table 4).

When patients experiencing NGU for the first time are considered, chlamydiae were isolated from

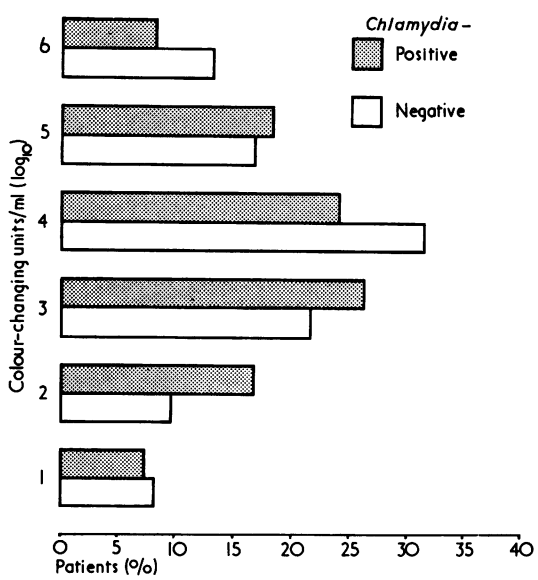

Fig. 1 *The quantitative distribution of ureaplasmas (titre expressed as log_{10} colour-changing units/ml) among chlamydia-positive and chlamydia-negative patients with NGU*

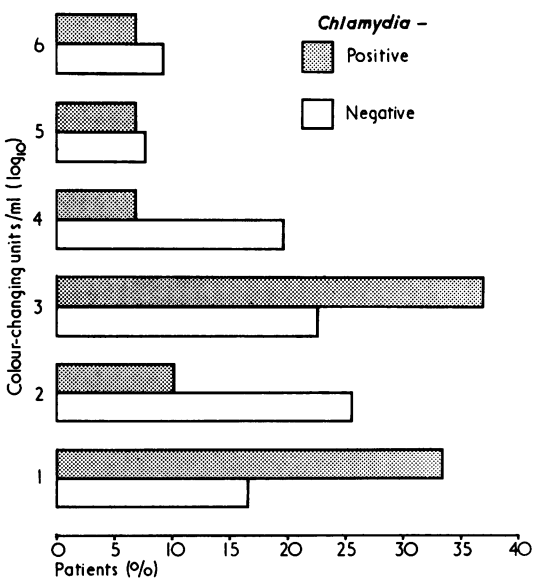

Fig. 2 *The quantitative distribution of* M. hominis *organisms (titre expressed as log_{10} colour-changing units/ml) among chlamydia-positive and chlamydia-negative patients with NGU*

Table 3 *Isolation of* Mycoplasma hominis *from patients who are and are not infected by* Chlamydia trachomatis *or by* Ureaplasma urealyticum

	% *of* M. hominis-*positive patients among those who are*					
	Chlamydia trachomatis			Ureaplasma urealyticum		
Hospital clinic	*Positive*	*Negative*	*Probability**	*Positive*	*Negative*	*Probability**
Central Middlesex	6·25	12·5	0·383	17·4	0	<0·005
Westminster	7·8	12·5	0·594	20·0	2·9	0·006
Shrodell (Watford)	7·9	11·8	0·725	20·3	6·3	<0·001
Central Middlesex	19·1	22·4	0·678	32·5	6·3	<0·005
St Mary's (Paddington)	6·25	9·1	0·932	16·1	0	<0·005
Total	11·4	14·5	0·284	22·9	2·6	<0·0005

*P is significant when <0·05

Table 4 *Isolation of* Chlamydia trachomatis *from patients who are and are not infected by* Ureaplasma urealyticum *or by* Mycoplasma hominis

	% *of chlamydia-positive patients among those who are*					
	Ureaplasma urealyticum			Mycoplasma hominis		
Hospital clinic	*Positive*	*Negative*	*Probability**	*Positive*	*Negative*	*Probability**
Central Middlesex	27·9	41·3	0·144	20·0	34·9	0·383
Westminster	47·3	36·8	0·318	30·8	42·7	0·594
Shrodell (Watford)	25·0	32·8	0·425	21·4	29·9	0·725
Central Middlesex	45·5	39·6	0·458	39·1	43·9	0·932
St Mary's (Paddington)	28·6	30·2	0·94	22·2	30·0	0·932
Total	35·9	36·5	0.762	30·9	37·0	0·284

*P is significant when <0·05

61 (40·4%) of 151 who were ureaplasma-positive and from 46 (40·3%) of 114 who were ureaplasma-negative, obviously an insignificant difference (χ_1^2 0·014, P=0·91). Likewise, in such patients chlamydiae were isolated from 16 (34·4%) of 47 who were *M. hominis*-positive and from 91 (41·7%) of 218 who were *M. hominis*-negative, also an insignificant difference (χ_1^2 0·659, P=0·41).

ASSOCIATION BETWEEN UREAPLASMAS AND *M. HOMINIS*

Ureaplasmas were isolated about twice as frequently from patients who were infected by *M. hominis* than from those who were not infected by this mycoplasma (Table 2), a difference which was significant for patients attending each clinic and for all patients (χ_1^2 62·56, P<0·0005). Conversely, *M. hominis* organisms were isolated more frequently from patients who were harbouring ureaplasmas than from those who were not (Table 3), the difference being significant for patients attending each clinic and for the total number of patients (χ_1^2 62·56, P<0·0005). The difference was also significant (χ_1^2 22·8, P<0.0005) for patients experiencing their first attack of NGU, *M. hominis* being isolated from 27·8% of those infected by ureaplasmas but from only 4·4% of those who were not.

Discussion

Several factors could lead to the isolation of ureaplasmas from patients with NGU and a failure to recover chlamydiae. Since the presence of epithelial cells in a urethral sample is more important for successful chamydial isolation than for ureaplasmal isolation, inadequate swabbing could result in a false predominance of ureaplasma isolates relative to chlamydiae. The same outcome could arise from an inefficient chlamydial isolation technique in the laboratory. We are aware, however, of these problems and consider that our chlamydial isolation technique, both in terms of specimen collection and laboratory procedures, has been satisfactory. Indeed, in studies over several years on a large number of men with NGU, our chlamydial isolation rate has been 36% for unselected patients and about 40% for patients experiencing their first attack. This is in accord with the results recorded by several other groups of workers (Wentworth, 1977). So far as ureaplasmas are concerned, we have found that swab specimens provide an estimate of the numbers of organisms present in the urethra comparable to that obtained by testing urine.

It is difficult to know whether the problems mentioned above could have led others to believe

that ureaplasmas are particularly associated with non-chlamydial NGU. Certainly, from both qualitative and quantitative viewpoints, we can find no basis for the association and we feel confident about this because such a large number of patients has been examined. It is also noteworthy that the data of Holmes *et al.* (1975) do not support the association. A significant association of ureaplasmas with chlamydia-negative NGU might be obscured by patients already possessing ureaplasmas, a situation more likely to occur in persons who have experienced multiple attacks of NGU or who have had multiple sexual partners than in those presenting with a first attack or who had had few sexual partners. We could not assess the influence that the number of sexual partners might have had on ureaplasma isolation, but by examining a large number of patients the unequal distribution among chlamydia-positive and chlamydia-negative groups of those who had, for example, a large number of sexual partners is likely to have been diminished. Certainly, examination of patients experiencing their first attack provided no evidence for the association of ureaplasmas with chlamydia-negative NGU. Furthermore, we have found that *M. hominis* organisms are not isolated more frequently from chlamydia-negative patients and that chlamydiae are not particularly associated with either ureaplasma positive or ureaplasma-negative patients or *M. hominis*-positive or *M. hominis*-negative patients. We have not been able to assess the numbers of chlamydial organisms infecting all the patients, but the qualitative results do not suggest that this would be worthwhile.

Apart from the technical reasons mentioned previously which might lead to false conclusions, one might consider whether it is reasonable for ureaplasmas or *M. hominis* to be particularly associated with chlamydia-negative patients. It is possible to argue that chlamydial damage to cells might provide a situation which is conducive to more prolific growth of ureaplasmas and *M. hominis*, as seen when certain viruses and mycoplasmas are mixed (Reed, 1971). This could lead to an association of ureaplasmas or *M. hominis* particularly with chlamydia-positive urethritis. Again, however, we have not found any evidence that this is so. Indeed, in a situation where there is an opportunity to acquire a variety of micro-organisms one must wonder why patients should be infected by one group of organisms to the exclusion of others. The only significant associations that we have noted are the more frequent isolation of *M. hominis* from men who are infected by ureaplasmas and the more frequent isolation of ureaplasmas from those who harbour *M. hominis*. This is an association which a number of other investigators, including, for example, Shepard *et al.* (1964) and Kundsin (1976), have remarked on or one which may be deduced from their data. Why this should be is difficult to understand and one can only surmise that the conditions within the genital tract are such that they favour the multiplication and establishment of both these micro-organisms and hence their association.

Our assertion that there is no greater association of ureaplasmas with chlamydia-negative than with chlamydia-positive NGU may raise further doubts in the minds of some workers about the pathogenicity of ureaplasmas. We do not believe, however, that the findings necessarily mitigate against these organisms being responsible for some cases of chlamydia-negative NGU. To propose this would seem as unreasonable as suggesting that chlamydiae have no part to play in nongonococcal disease because they have been isolated by some investigators, such as Richmond *et al.* (1972) and Ridgway and Oriel (1977), as frequently from patients with gonococcal disease as from those with nongonococcal disease. It is equally clear, however, that the present results do not allow us to consider that all non-chlamydial urethritis is ureaplasmal. Indeed, the failure to recover ureaplasmas from about 50% of patients with non-chlamydial NGU means that in about 30% of patients with NGU neither of these micro-organisms can be recovered. Unless the present chlamydial and ureaplasmal isolation rates are eventually proved to have been underestimated, this suggests that these cases have another aetiology.

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